IN THE FIGURES:

Replacement sheets using the correct "SEQ ID NO:" indicator are attached to this amendment.

REMARKS

Status of Claims

Claims 1-59 were pending.

Claims 11 and 38 (said mammal is human) have been incorporated into claims 2 and 30. Claims 11 and 38 are thus canceled.

Claims 57-59 are also canceled

Claim 60 is added to recite a second species of preferred sequences.

In response to Applicant's election of Group 2 in a previous restriction requirement, the Examiner indicates that claims 2 and 13 are under examination and that claims 1, 3-12, and 14-56 were withdrawn from examination.

Applicants submit that, in view of the amendment of dependent claims 4-7, 9-10, 12 and 15-29 to depend from claim 2, and in view of addition of claim 60 depending from claim 2, these dependent claims are now also within the elected invention, and under examination.

Accordingly, the claims under examination on the merits are: 2, 4-7, 9-13, 15-29 and 60.

Further, Applicants submit that the claims as presently amended clearly avoid the prior art which was the basis for the determination of "lack of common technical feature" in the Lack of Unity of Invention determination in the WO application. The present claims (a) testing of a body fluid sample and not a tissue sample, (b) perform a diagnostic test rather than monitoring progression of gene regulation during the course of sepsis, and (c) allow treatment of live humans rather than involving sacrificed rat. Since all claims are now directed to the same patentable invention and common technical feature and unity of invention having been established, claims 1, 3, 8, and 14, at a minimum (quantifying gene levels in body fluid samples), should be rejoined, and in fairness all claims (including claims 30-56 directed to quantifying protein levels of body fluid samples) should be under examination as being directed to the same generic invention.

Summary of Interview

The Interview Summary indicates "the formal written reply to the last Office Action must include the substance of the interview – unless Examiner initiated interview.

In response, Applicants indicate that the interview was Examiner initiated, they agree with the summary set forth in the Interview Summary Continuation Sheet and associated text regarding separating two groups of species, have added only the elected species to claim 13, and added a new claim 60 depending from claim 2 reciting the second set of sequences.

"New" Restriction Requirement

In the original Restriction Requirement the Examiner characterized the "common technical feature" of the invention as use of particular genes associated with various sepsis related conditions.

In response, Applicants pointed out that the invention does not involve screening for particular genes, but rather, is characterized by quantitative testing, measuring LEVELS of genes.

In the present Office Action the Examiner takes the position that measurement of levels of gene expression in the analysis of sepsis and inflammation was known in the prior art at the time the invention was made (i.e. as taught in Chinnaiyan et al (1999) cited in the Lack of Unity Requirement for Restriction of 08/20/2008). According to the Examiner, the asserted "common technical feature" is not a special technical feature in view of the prior art, and thus does not provide unity of invention.

Applicants respectfully submit that the claims, as amended to require use of body fluids for measurement of gene expression profiles for diagnosis (specification, paragraph [0025]), and further requiring quantitative detection, defines a common and patentable technical feature.

As recited in paragraph [0026]: "The point of origin of the invention disclosed in the present patent application is the realization that RNA levels different from normal values respectively peptide levels or peptide segment levels derivable from the RNA levels, that can be detected in a serum or plasma of a patient whose risk is high that he will be suffering from SIRS,

or who suffers from symptoms that are typical for SIRS, can be detected before SIRS, sepsis, sepsis-like conditions, severe sepsis and systemic Infections are detected in biological samples."

Basically, Chinnaiyan harvest organs at various stages in development of sepsis and screen DNA using a 8064 element microchip, identifying which genes are "up regulated" and which genes are "down regulated", and analyze the patterns of gene regulation in an attempt to elucidate the mechanism of the pathogenesis of sepsis. Chinnaiyan have no teaching relevant to the present invention, which provides a tool for early diagnosis of sepsis by measuring levels of expression of genes in bodily fluids of a patient (a mammal).

More specifically, Chinnaiyan discuss that, in surgical and medical intensive care units, sepsis often leads to pulmonary, hepatic and renal failure, this triad commonly being referred to as "multiorgan failure syndrome." "Although this pattern of organ failure is well-known clinically, its pathogenesis is poorly understood." (Discussion) Chinnaiyan "examined the temporal sequence of sepsis-induced gene expression patterns in major organ systems including lung, liver, kidney, thymus, spleen, and brain" (Abstract) in an attempt to elucidate the mechanisms of sepsis.

Chinnaiyan also explain usefulness of DNA microarray for testing many different genes at the same time (paragraph bridging pages 1199 and 1200), and explain that they developed an 8064 glass slide element rat cDNA microarray including ~2000 known, named genes to analyze multiorgan/multisystem gene expression patterns (page 1200, first col., last para., and results, first para.). "We propose that the response to sepsis induces both distinct and shared gene expression programs in various organs... Our hypothesis is that microarray analysis of genes expressed in organs during sepsis may be predictive of outcome, especially in organs that are known to be compromised during sepsis. Such studies may provide important insight into multiorgan failure during sepsis. Although several studies have successfully used DNA microarrays to molecularly classify malignancies, this is the first gene-profiling study to address an important disease process at a multiorgan, multisystem level."

Thus, Chinnaiyan hypothesize that by identifying via microarray analysis which genes are expressed during course of development of sepsis, they may better understand the mechanisms of sepsis. There is no hint as to relevancy of levels of expression of genes.

Chinnaiyan do not suggest a prognostic test to be conducted with living patients. Rather, they teach post mortem analysis of organs harvested at various times post-op to attempt to reconstruct disease progress over time at a multi-organ, multi-system level. "Lung, liver, thymus, spleen, kidneys, and brain were harvested from CLP rats, sham-rats, and control untreated rats. Various time points (6, 12, 18, and 24 hours) after surgery were used in the CLP and sham animals. Organs from three rats from each condition were pooled, snap-frozen, and stored at -80°C." (Materials and Methods) Such teaching is hardly suggestive of or applicable to diagnostic and preventative methods of the present invention.

Under *Microarray Analysis* Chinnaiyan teach DNA microarray analysis of gene expression. This test is a screening test, for identification of genes, not for quantification of levels of expression. Note that the microchips include various control elements. None of the control elements were designed for calibration or measurement of levels of expression. Test were carried out on pooled rat organs. Fluorescent images of hybridized microarrays were obtained using a GenePix 4000A microarray scanner (www.axon.com; Axon Instruments, CA).

As reported in *Data Analysis,* fluorescent images of hybridized microarrays were analyzed and genes were select as significantly up- or down-regulated relative to the control sample. The data sets for each organ were individually queried for genes that were differentially expressed in the CLP organs as compared to control organs, i.e, ratios >2.0 for up-regulated, or <0.5 for down-regulated. Thus, Chinnaiyan are merely interested in identifying which genes are turned on, i.e., exceed or fall be low a threshold, and not the level of gene expression.

"[W]hen organs from septic animals were compared to respective control organs, differential gene expression was observed" (Fig. 1). "Gene expression was monitored in six organs at various times points (6, 12, 18, and 24 hours) in the CLP rat model. Hierarchical clustering of the data identified distinct patterns of gene expression in the organs studied. Red

and green colors in the matrix represent genes that are up- and down-regulated, respectively, relative to the untreated control organ." (Fig. 2) Again, patterns of identified genes, not levels of gene activation, are studied. The patterns of gene expression in each organ are compared.

The section *Validation of Selected Genes Identified by Microarray Analysis* discusses levels of expression, but only as part of validation check. There is no suggestion that expression levels have any relevancy to sepsis.

Finally, by identifying which genes are regulated, Chinnaiyan identify or at least postulate a pathway of sepsis. "Thus, our data suggest that several tissues mobilize downstream components of the IL-6 signaling pathway in response to sepsis and are presumably primed for activation by IL-6." (*Functional Analysis of Sepsis-Induced Gene Expression Patterns)*

Accordingly, as Chinnaiyan does not anticipate or render obvious the present claims, as amended, it is respectfully submitted that the restriction requirement should be withdrawn and all claims should be reunited for examination.

Finally, the Examiner indicates that "while Applicants submit that the inventions of claims 2, 3, 13, and 14 would be obvious over each other, the express admission of obviousness is noted; but in the instant case the application is a national stage application of a PCT where Lack of Unity rules apply to separation of different inventions, and the Examiner maintains that the different invention of the claims are properly separable under Lack of Unity rules."

In response, applicants point out that the present claims as amended do not suffer from lack of unity, thus are properly united into one application, with or without the admission.

Election of Species Requirement

The Examiner takes the position that Applicant's further election with traverse of the invention of particular combination of sequences of those sequences recited on pages 13-14 of the Remarks with the 'Patent Seq ID' of Roman numeral 'I' is acknowledged. The traversal is on the ground(s) that 'sepsis conditions are determined not by qualitative measurement of any particular genes, but quantitative measurement. The Examiner does not find this to be persuasive

because, regardless of the type of measurement (i.e. qualitative or quantitative) the different nucleic acid sequences are structurally distinct elements, as set forth on page 3 of the Requirement of 08/20/2008.

Applicants respectfully traverse. Just as a patent on a method of making an apple pie is not properly restricted due to use of separate ingredients such as flour, sugar and apples, a method for detection of sepsis based on level of gene expression is not properly restricted to the individual genes in the gene pool being tested.

Accordingly, withdrawal of the election of species in view of the overriding unifying inventive concept is respectfully requested.

Priority

The Examiner indicates that the priority documents have not been received by the US Receiving Office.

In response, Applicants have verified that such documents have been supplied to the International Bureau. Applicants have sent a letter to the International Bureau requesting that these documents be electronically transferred to the US Receiving Office.

Should these documents not arrive by the time of the issuance of the Notice of Allowance, Applicants will obtain new certified copies of the priority documents.

Objection to the claims and Specification - Sequence Compliance

According to the Examiner, this application contains references to sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825.

In response, the specification, claims and figures have been amended to the proper format for the sequence identifier as set forth in 37 CFR 1.821.

Specification

The disclosure is objected to because of the following informalities:

In the specification, ¶130 references Tables 2 and 3, where likely reference to Tables 8 and 9 is intended. Applicants should review ¶130 to ensure that the appropriate tables are referenced.

Applicants note that paragraph [0130] of the application corresponds to paragraph [0168] of the specification as published. Applicants have amended paragraph [0168] of the specification as published.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. See for example pages 22, 26, 31, and 42. Applicant is required to delete the embedded hyperlink and/or other form of browser- executable code from throughout the specification. See MPEP § 608.01.

In response, Applicants direct readers to the web site for the National Center for Biotechnology Information:

[0148] In the appended sequence listing, which is part of the present invention, the gene bank accession numbers indicated in tables 2 and 3 (access via internet via the web site for the National Center for Biotechnology Information ~~http://www.ncbi.nlm.nih.gov/~~) of the individual sequences are each allocated to one sequence ID.

Claim Objections

Claim 2 is objected to over recitation of the phrase 'isolating of sample RNA from a sample of a mammal', where the phrase 'isolating sample RNA from a sample from a mammal' is correct.

In response, Applicants appreciate the guidance, and have amended all to be consistent with US practice.

Claim 13 is objected to for the specific recitation of non-elected subject matter.

In response, Claim 13 has been amended to be limited to the elected combinations of genes with the sequences as set forth in the first 57 sequences identified in the Table on pages 13-14 of the Remarks of 11/20/2008.

Applicants appreciate that, upon allowance of a claim directed to the elected invention, the Examiner may consider rejoinder of the subject matter of the non-elected combinations, and rejoinder of any combinations that include all of the limitations of the allowed elected sub-combination.

Claim Rejections - 35 USC § 112 2nd ¶ Indefiniteness

Claims 2 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

According to the Examiner, claims 2 and 13 are unclear over the purpose of the claimed methods 'for in vitro detection of sepsis and/or sepsis-like conditions' as recited in the preamble of claim 2. Claim 2 recites the methods steps of the claim as isolating and labeling RNA from a sample, contacting RNA with DNA, and detecting and comparing label signals. The final active process step is comparing label signals. There is no active step in which sepsis or a sepsis-like condition is in fact detected. Thus there is not a nexus between the purpose of the claimed method as stated in the preamble of claim 2 and the methods steps, and it is unclear how the performance of the methods steps results in the required 'detection of sepsis and/or sepsis-like conditions'.

In response, Applicants amend the independent method claims to recite the object of the method and the positive steps which result in the accomplishing of the object.

Claims 2 and 13 are unclear over recitation of the phrase 'contacting the sample RNA with the DNA under hybridization conditions' because, with regard to the phrase 'the DNA' the only prior recitation of 'DNA' is in the alternative 'sample RNA and/or at least one DNA'. Thus there is no definitive requirement for DNA and there is a lack of sufficient antecedent basis for

'the DNA' in the phrase 'contacting the sample RNA with the DNA under hybridization conditions'. See MPEP 2173.05(e).

All claims have been amended to correct the antecedent basis issue.

Claim 13 is unclear over recitation of the phrase 'the gene or gene segment' because there is a lack of sufficient antecedent basis for any 'gene segment'. See MPEP 2173.05(e).

All claims have been amended to correct the antecedent basis issue.

Claim Rejections - 35 USC § 112 1st Para- Written Description

Claims 2 and 13 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The rejection of claims for lack of adequate written description is relevant to the requirement of the claims, drawn to methods for in vitro detection of sepsis and/or sepsis-like condition, for RNA or DNA ***'being a gene or gene fragment specific for sepsis'*** *(as recited in claim 2), and* ***'gene fragments thereof' with as few as 5 nucleotides*** *(as recited and encompassed by claim 13). In the instant case the specification does not provide the skilled artisan with an adequate written description of particular nucleic acids suitable for performing the claimed method as generically encompassed by the claim 2 and minimally comprising 5 nucleotides as encompassed by claim 13.*

In response, Applicants refer the Examiner to the specification, paragraph [0028]: "The method of the invention is characterized in that the activity of one or more leading genes can be determined in a sample of a biological liquid of an individual. Additionally, SIRS and/or the success of a therapeutic treatment can be deduced from the presence and/or, if present, the amount of the determined gene product." (emphasis added) As explained in paragraph [0168] "Table 8 shows that 230 genes of the patient sample were found, which were ***significantly***

overexpressed (expression ratios between 13.67 and 98.33), if compared with the control sample. Table 9 further shows that 206 genes of the patient sample were found, which were ***significantly*** underexpressed (expression ratios between 0.01 and 0.09), if compared with the control sample. Those results show that the genes listed in Tables 8 and 9 correlate with the occurrence of SIRS. Thus, the genes mentioned are leading genes for the diagnosis of an early sepsis." As explained in paragraph [0145] of the specification: "[0145] Table 2 shows that 57 genes of the patient sample were found, which were ***significantly*** overexpressed, if compared with the control sample. Table 3 shows that 16 genes of the patient sample were found, which were ***significantly*** underexpressed, if compared with the control sample. Those results show that the genes listed in table 2 and table 3 correlate with the occurrence of SIRS. Thus, the gene activities of the genes mentioned are labels for a diagnosis of SIRS."

The present invention is accomplished by a test for significant overexpression of at least one specified gene, and for improved reliability, overexpression of more than one of the identified genes.

The Examiner indicates that genes, the expression of which is diagnostically indicative of sepsis or sepsis-like conditions, are not known in the prior art.

Applicants respectfully traverse. As indicated in the specification, paragraph [0022], "it is known from WO 03/002763 that microarrays basically can be used for the diagnosis of sepsis and sepsis-like conditions." Further, Chinnaiyan identify which genes are up- and down-regulated, and use this information to postulate a pathway of sepsis. See particularly the sections *Clustering of Gene Expression Patterns Induced by Sepsis* and *Functional Analysis of Sepsis-Induced Gene Expression Patterns* and associated figures.

Thus,

- from WO 03/002763 specific genes are known which are indicative of sepsis,
- Chinnaiyan specific genes are known, they are just extracted from tissue samples rather than body fluids, and

- from the present specification one finds a listing of genes of which overexpression or underexpression is indicative of sepsis, etc.

Further, while the specification asserts that there is a group of gene from humans that differentially expresses in humans with sepsis as compared to a non-septic individual (i.e. Tables 8 and 9), there is no disclosed relationship between ***the structure*** of the genes (i.e. their nucleotide sequences) and ***their functionality*** (i.e. diagnostic of sepsis). This is also relevant to the breadth of claim 13, which, consonant with the election, which encompasses any genes comprising as few as 5 nucleotides of the mRNA sequences as elected.

In response, Applicants point out that sepsis is a complex process involving many genes as explained in Chinnaiyan. If there were a relationship between structure and function, then the invention, and the claims, could be greatly simplified. However, there is no such simple relationship of structure and function, as found in cases of chemical analogy. Thus, the present invention requires listing of genes which when overexpressed are indicative of the sepsis condition.

The Examiner then indicates that, having considered the breadth of the claims, and the particular teachings of the instant specification, and the teachings of the prior art, it is the opinion of the Examiner that the specification, while providing a written description of methods requiring the step of, for example comparing the abundance of particular mRNA species from a sample to the abundance of the same mRNA species from a control sample, wherein the mRNA are those listed in current claim 13, it does not provide an adequate written description of the broadly claimed subject matter.

In response, Applicants submit that the specification teaches a great variety of species of genes which are suitable indicators of sepsis or other recited condition, that these species are united as indicators of sepsis when highly expressed, and that the Examiner should thus withdraw the restriction requirement and allow consideration of all species within the present application.

Claim Rejections - 35 USC § 112 1st j - Enablement

Claims 2 and 13 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention:

Nature of the invention and breadth of the claims

The claims are drawn to methods of detection of sepsis and/or sepsis like conditions.

The claims encompass the analysis of any subject mammal.

The claims generically encompass analysis of any gene or fragment specific for sepsis.

The claims encompass any comparison of any labeled RNA, as well as any fragments of the elected combination of SEQ ID NOs.

The claims encompass detection of any condition that can be considered a `sepsis-like condition'. The claims thus require knowledge of a correlative association between any expression levels of a wide variety of RNA combinations and a variety of different phenotypes in different subjects.

In response, Applicants note that the Examiner has found the written description to be satisfied for claim 13, as amended. Applicants would be pleased to recite all genes listed in the specification in the claims, if the Examiner would permit this by removing the restriction requirement.

Direction provided by the specification and working example

*Relevant to the Election, the instant specification provides a comparative analysis (Example 3 — p.26) of gene expression in two **human** individuals, one classified as a sepsis patient and the other classified as a non-septic control subject (Table 7). The specification asserts that 54 particular genes were overexpressed in the sepsis-patient sample (Table 8), and 56 particular gene were under-expressed in the sepsis-patient sample (Table 9).*

Relevant to the claims and the elected invention, the specification provides the aforementioned analysis of sepsis gene expression, but does not provide for gene expression in the generic ***'sepsis-like'*** *conditions.*

In response, Applicants explain that in hospital practice sepsis is diagnosed if two of the SIRS criteria are fulfilled and a systemic bacterial or fungal infection is detected. Such an infection is only detected in 10% to 15% of all blood cultures, as the methods available prior to the present invention are unsatisfactory. In most cases, sepsis is diagnosed only based on symptoms and the clinical appearance. In other words, sepsis is often diagnosed based on subjective evaluation rather than objective criteria. Thus, sepsis is often assumed but not diagnosed conclusively. From this clinical point of view ***only "sepsis-like conditions" may be diagnosed***. Precisely this problem is solved by the present invention, for which a robust, reliable and objective criterion for the diagnosis of sepsis is provided.

The detection of RNA from body fluids (e.g. blood, urine, saliva) according to the present invention as currently claimed is considerably more advantageous than the detection of RNA form tissue samples. Samples may be isolated easier, i.e. in a non-invasive way, from body fluids. Samples from tissue samples can only be isolated by means of a biopsy which is an invasive method.

For a reliable diagnosis it is basically important that the change of the gene expression level is reproducible and clearly recognizable. Thus, genes which show considerable overexpression may be well combined with genes which show clear underexpression. Normally, fragments of a selection of genes determined as being over- and underexpressed in case of a specific disease (e.g. genes of Tables 2 and 3 for SIRS) will be immobilized on a chip. The number of genes needed for a reliable diagnosis varies (often between 2 and 14).

A possibility for diagnosis is to contact such a chip with the labelled total RNA (the whole fragmented genome or all genes) isolated from a healthy subject and contact a second identical chip with the labelled total RNA isolated from a subject suspected of having SIRS. Only those (few) genes of the total RNA will bind whose corresponding (complementary) fragments are immobilized on the chip, the rest will be washed away.

The specification does not provide any analysis of any other non-human subject organisms.

The specification provides only the results of a comparison between two individual subjects (a single case and a single control), with no validation of the asserted particular mRNAs specific for sepsis, nor any analyses of populations of cases or controls. There is no statistical analysis of the reliability of classification using expression of particular mRNA species.

Additionally it is noted that the particular mRNAs asserted in the specification (Tables 8 and 9) to be indicative of sepsis are not included in the particular mRNAs of the Election.

In response, Applicants submit that the human is a representative test animal for the condition of sepsis in mammals in general, and having proven the early detection in humans, have provided the ultimate in evidence of detection in mammals. It is not necessary that experiments be conducted with all mammals.

Nevertheless, to expedite allowance, Applicants have limited all claims to human.

State of the art, level of skill in the art, and level of unpredictability

While the state of the art and level of skill in the art with regard to determining the abundance of any particular nucleic acid biomarker or combination of biomarkers is high, the unpredictability associated with correlating any comparison of abundances with a particular phenotype such as sepsis, is even higher. Such unpredictability is demonstrated by the prior art, the post-filing art, and the instant specification.

Because the claims encompass the analysis of biomarkers from any subject mammal, whereas the instant specification provides only an anlysis of human subjects, it is relevant to point out the unpredictability in extrapolating gene expression results among different organisms. Such unpredictability is exemplified by Hoshikawa et al (2003), which teaches unpredictability with regard to applying gene expression results among different organisms. The reference teaches the analysis of gene expression in lung tissue in response to hypoxic conditions which lead to pulmonary hypertension (Fig. 1). The reference teaches that the gene expression

profile in mouse is different from that observed in rat (Tables 1-4; p.209 - Abstract). Thus it is unpredictable as to whether or not any genes that are sepsis-related in, for example, human are in fact applicable to predicting sepsis in any other non-human organism.

Because the claims encompass comparing any abundances of any particular RNAs to any control RNAs, where the specification provides only the example of analysis of two individuals (one case and one control), it is relevant to point out the unpredictability in using gene expression to establish a phenotype. For example, Cheung et al (2003) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) (p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or areater (Fig 3). Additionally, the prior art of Shalon et al (2001) teaches that preferably 20-50 different test individuals are assayed to obtain meaningful data showing a significant change in gene expression levels, and changes of gene expression of at least 2 fold and up to 100 fold or more are desirable for the comparison of gene expression levels between a case and control population (p.10 11156, ¶158). Further, it is known in the art that the p-value of any marker used to diagnose sepsis will change based on the size of the population used for comparison (PG Pub US 2004/0106142, p.14, 11[0127]).

Given the lack of any statistical significance in the methods, it is relevant to point out that the prior art of Thisted (1998) provides guidance as to what is required to indicate that an association is statistically significant (Thisted teaches that it has become scientific convention to say that a P-value of 0.05 is considered significant (p.5 - What does it mean to be 'statistically significant), and that values above the conventional reference point of 0.05 would not be considered strong enough for the basis of a conclusion).

*Because claims encompass the analysis of gene expression in **any tissue types**, whereas the specification provides only expression in whole blood samples, it is relevant to point out the unpredictability in comparing gene expression among different tissues. Cobb et al (2002) teaches the unpredictability in analysis of gene expression different tissues of a septic mammal, specifically in spleen and liver samples from septic mice. Notably, the reference teaches that, when compared to a non-septic sample, the relevant expression profiles of the septic mouse **spleen** and the septic mouse **liver** contain different nucleic acids at different levels (Table 1; p.2714, middle col., lns.2-8).*

In response, Applicants point out that the claims have been limited to human, and

- the specification provides highly statistically relevant correlation between gene expression and condition of sepsis. As indicated in paragraph [0168], Table 8 shows that 230 genes of the patient sample were found, which were ***significantly*** overexpressed (expression ratios between 13.67 and 98.33), if compared with the control sample. Table 9 further shows that 206 genes of the patient sample were found, which were ***significantly*** underexpressed (expression ratios between 0.01 and 0.09), if compared with the control sample. Those results show that the genes listed in Tables 8 and 9 correlate with the occurrence of SIRS. Thus, the genes mentioned are leading genes for the diagnosis of an early sepsis. As explained in paragraph [0145], Table 2 shows that 57 genes of the patient sample were found, which were ***significantly*** overexpressed, if compared with the control sample. Table 3 shows that 16 genes of the patient sample were found, which were ***significantly*** underexpressed, if compared with the control sample. Those results show that the genes listed in table 2 and table 3 correlate with the occurrence of SIRS. Thus, the gene activities of the genes mentioned are labels for a diagnosis of SIRS; and
- the claims as amended are directed to body fluid samples, thus provide a simple early diagnostic test different from a tissue sample.

Quantity of experimentation required

A large and prohibitive amount of experimentation would be required to make and use the claimed invention. One would have to establish that any level of nucleic acid abundance of ***any RNAs****, as compared to value in any control, is indicative of sepsis. Such experimentation would require case:control analysis of any subject mammal of interest, and require the analysis of different tissue types and analysis of any RNA species of interest. Even for the particularly elected SEQ ID NOs it is noted that the instant specification does not provide that these mRNAs are robustly and reliably diagnostic of the presence of sepsis or any other condition that may be considered 'sepsis-like'.*

In response, if the Examiner is willing to allow claim 13 to detection of sepsis in humans, and accepts the rational for inclusion of the term "sepsis like condition" in the claims, then Applicants should be entitled to examination of additional species of genes. Applicants thus request the Examiner to withdraw the election of species requirement

Conclusion

Taking into consideration the factors outlined above, including the nature of the invention and breadth of the claims, the state of the art, the level of skill in the art and its high level of unpredictability, the lack of guidance by the applicant and the particular examples, it is the conclusion that an undue amount of experimentation would be required to make and use the claimed invention.

In response, enabling teaching having been found for claim 13, it is respectfully requested that other species claims of similar scope and enablement also be indicated to be allowable.

Claim Rejections - 35 USC § 102

It is noted that claim rejected under 35 U.S.C. 102 as anticipated by the prior art have been previously rejected under 35 USC 112 1st ¶ for lack of enablement.

Claim 2 is rejected under 35 U.S.C. §102(b) as being anticipated by Chinnaiyan et al (2001) (as cited on the IDS of 3/15/2006). Chinnaiyan et al teach the analysis of gene expression in a sepsis model. Relevant to the rejected claims, the reference teaches isolating a sample of RNA from the subject mammal and labeling the sample RNA, contacting the sample RNA with DNA under hybridization conditions and analysis of RNA from a non-pathological control, and quantitative detection of labels (p.1200 - Rat model of sepsis; Microarray analysis). The microarray analysis of the reference comprises comparing the subject and control signals to determine expression levels as compared to each other (e.g. Fig. 2).

In response, Applicants refer the Examiner to the above extensive discussion of Chinnaiyan et al. In view of the amendment of the present claims to body fluid samples from human, and in view of the technical distinctions listed above, the present claims are not anticipated by this reference.

As a general explanation as to the importance of the present claim limitations, in hospital practice sepsis is diagnosed if two of the SIRS criteria are fulfilled and a systemic bacterial or fungal infection is detected. Such an infection is only detected in 10% to 15% of all blood cultures, as the methods available prior to the present invention are unsatisfactory. In most cases, sepsis is diagnosed only based on symptoms and the clinical appearance. In other words, sepsis is often diagnosed based on subjective evaluation rather than objective criteria. Thus, sepsis is often assumed but not diagnosed conclusively. From this clinical point of view only "sepsis-like conditions" may be diagnosed. Precisely this problem is solved by the present invention, for which a robust, reliable and objective criterion for the diagnosis of sepsis is provided.

The detection of RNA from body fluids (e.g. blood, urine, saliva) according to the present invention as currently claimed is considerably more advantageous than the detection of RNA form tissue samples. Samples may be isolated easier, i.e. in a non-invasive way, from body fluids. Samples from tissue samples can only be isolated by means of a biopsy which is an invasive method.

For a reliable diagnosis it is basically important that the change of the gene expression level is reproducible and clearly recognizable. Thus, genes which show considerable

overexpression may be well combined with genes which show clear underexpression. Normally, fragments of a selection of genes determined as being over- and underexpressed in case of a specific disease (e.g. genes of Tables 2 and 3 for SIRS) will be immobilized on a chip. The number of genes needed for a reliable diagnosis varies (often between 2 and 14).

A possibility for diagnosis is to contact such a chip with the labelled total RNA (the whole fragmented genome or all genes) isolated from a healthy subject and contact a second identical chip with the labelled total RNA isolated from a subject suspected of having SIRS. Only those (few) genes of the total RNA will bind whose corresponding (complementary) fragments are immobilized on the chip, the rest will be washed away.

As a reference or for correct comparison of the hybridisation results also genes may be immobilized on the chip which show the same expression level in healthy subjects as well as in subjects with SIRS. When comparing both chips, differences in expression will be observed in case the ill patient has SIRS.

Turning to Chinnaiyan, this reference teaches to harvest organs at various stages in development of sepsis and screen DNA using a 8064 element microchip, identifying which genes are "up regulated" and which genes are "down regulated", and analyze the patterns of gene regulation during development of sepsis in an attempt to elucidate the mechanism of the pathogenesis of sepsis. Chinnaiyan have no teaching relevant to the present invention. The present invention provides a tool for early diagnosis of sepsis by measuring levels of expression of genes in bodily fluids of a patient (a mammal, preferably a human).

More specifically, Chinnaiyan discuss that, in surgical and medical intensive care units, sepsis often leads to pulmonary, hepatic and renal failure, this triad commonly being referred to as "multiorgan failure syndrome." "Although this pattern of organ failure is well-known clinically, its pathogenesis is poorly understood." (Discussion) Chinnaiyan "examined the temporal sequence of sepsis-induced gene expression patterns in major organ systems including lung, liver, kidney, thymus, spleen, and brain" (Abstract) in an attempt to elucidate the mechanisms of sepsis.

Chinnaiyan also explain usefulness of DNA microarray for testing many different genes at the same time (paragraph bridging pages 1199 and 1200), and explain that they developed an

8064 glass slide element rat cDNA microarray including ~2000 known, named genes to analyze multiorgan/multisystem gene expression patterns (page 1200, first col., last para., and results, first para.). "We propose that the response to sepsis induces both distinct and shared gene expression programs in various organs... Our hypothesis is that microarray analysis of genes expressed in organs during sepsis may be predictive of outcome, especially in organs that are known to be compromised during sepsis. Such studies may provide important insight into multiorgan failure during sepsis. Although several studies have successfully used DNA microarrays to molecularly classify malignancies, this is the first gene-profiling study to address an important disease process at a multiorgan, multisystem level."

Thus, Chinnaiyan hypothesize that by identifying via microarray analysis which genes are expressed during course of development of sepsis, they may better understand the mechanisms of sepsis. There is no hint as to relevancy of levels of expression of genes.

Chinnaiyan do not suggest a prognostic test to be conducted with living patients. Rather, they teach post mortem analysis of organs harvested at various times post-op to attempt to reconstruct disease progress over time at a multi-organ, multi-system level. "Lung, liver, thymus, spleen, kidneys, and brain were harvested from CLP rats, sham-rats, and control untreated rats. Various time points (6, 12, 18, and 24 hours) after surgery were used in the CLP and sham animals. Organs from three rats from each condition were pooled, snap-frozen, and stored at -80°C." (Materials and Methods) Such teaching is hardly suggestive of or applicable to diagnostic and preventative methods of the present invention.

Under *Microarray Analysis* Chinnaiyan teach DNA microarray analysis of gene expression. This test is a screening test, for identification of genes, not for quantification of levels of expression. Note that the microchips include various control elements. None of the control elements were designed for calibration or measurement of levels of expression. Test were carried out on pooled rat organs. Fluorescent images of hybridized microarrays were obtained using a GenePix 4000A microarray scanner (www.axon.com; Axon Instruments, CA).

As reported in *Data Analysis,* fluorescent images of hybridized microarrays were analyzed and genes were select as significantly up- or down-regulated relative to the control sample. The data sets for each organ were individually queried for genes that were differentially

expressed in the CLP organs as compared to control organs, i.e, ratios >2.0 for up-regulated, or <0.5 for down-regulated. Thus, Chinnaiyan are merely interested in identifying which genes are turned on, i.e., exceed or fall be low a threshold, and not the level of gene expression.

"[W]hen organs from septic animals were compared to respective control organs, differential gene expression was observed" (Fig. 1). "Gene expression was monitored in six organs at various times points (6, 12, 18, and 24 hours) in the CLP rat model. Hierarchical clustering of the data identified distinct patterns of gene expression in the organs studied. Red and green colors in the matrix represent genes that are up- and down-regulated, respectively, relative to the untreated control organ." (Fig. 2) Again, patterns of identified genes, not levels of gene activation, are studied. The patterns of gene expression in each organ are compared.

The section *Validation of Selected Genes Identified by Microarray Analysis* discusses levels of expression, but only as part of validation check. There is no suggestion that expression levels have any relevancy to sepsis.

Finally, by identifying which genes are regulated, Chinnaiyan identify or at least postulate a pathway of sepsis. "Thus, our data suggest that several tissues mobilize downstream components of the IL-6 signaling pathway in response to sepsis and are presumably primed for activation by IL-6." (*Functional Analysis of Sepsis-Induced Gene Expression Patterns).*

Accordingly, as Chinnaiyan does not anticipate or render obvious the present claims, as amended, it is respectfully submitted that the restriction requirement should be withdrawn and all claims should be reunited for examination.

The "common inventive feature" of the present invention is that the present invention is based on the discovery that diagnosis can be made using body fluids and detecting

- RNA levels different from normal levels, or
- RNA derived peptide levels or
- peptide segment levels derivable from RNA levels.

This is a departure from Chinnaiyan et al using microarrays to screen for the up regulation or down regulation of genes from tissue samples in sacrificed rats. In the present invention, in contrast, to differentiate between symptoms that are based on microbial infections and other symptoms of non-infectious etiology, which could indicate sepsis due to their **clinical**

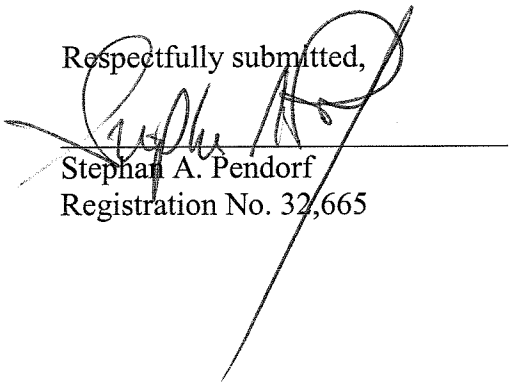
appearance, but are in fact not based on a systemic microbial infection, e.g. of symptoms that base on non-infectious inflammation of individual organs, the determination of gene expression profiles via differential diagnostics proved to be particularly advantageous. The use of body fluids for the measurement of gene expression profiles for the diagnosis of SIRS has however not yet been described. The point of origin of the invention disclosed in the present patent application is the realization that **RNA levels** different from normal values respectively **peptide levels or peptide segment levels** derivable from the RNA levels, that can be detected in a serum or plasma of a patient whose risk is high that he will be suffering from SIRS, or who suffers from symptoms that are typical for SIRS, can be detected before SIRS, sepsis, sepsis-like conditions, severe sepsis and systemic Infections are detected in biological samples.

Withdrawal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any fees which may be required at any time during the prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account Number 16-0877.

Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.

Respectfully submitted,



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